Connective Tissue Metabolism in Patients with Unclassified Polyarthritis and Early Rheumatoid Arthritis. Relationship to Disease Activity, Bone Mineral Density, and Radiographic Outcome

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ABSTRACT. ***Objective.*** To assess the applicability of serum concentrations of markers of synovial inflammation, cartilage, and bone metabolism in relation to conventional markers of disease activity, bone mineral density (BMD) of the hand, and radiographic outcome.

Methods. Biochemical markers of collagen tissue metabolism were measured in 72 patients with symmetrically swollen and tender second and third metacarpophalangeal or proximal interphalangeal joints for at least 4 weeks and less than 2 years. At 2 years, 51 patients fulfilled the American College Rheumatology criteria for rheumatoid arthritis (RA) and 21 patients had unclassified polyarthritis. Patients with RA were divided into groups according to the mean disease activity and to magnetic resonance imaging and radiographically detected bone erosions in the hands.

Results. Patients with RA had significantly higher serum concentrations of matrix metalloproteinase-3 (MMP-3) at baseline and higher mean concentrations of serum MMP-3 and pyridinoline (Pyd) during the first 6 and 12 months than patients with unclassified polyarthritis. RA patients with persistent disease activity and erosive disease had significantly higher concentrations of serum MMP-3 and Pyd than patients with no disease activity or nonerosive disease. Significant mutual correlations between serum MMP-3 and Pyd and C-reactive protein and erythrocyte sedimentation rate were observed. The mean values of MMP-3 and Pyd correlated significantly to the alpha coefficient of the digital x-ray radiogrammetry (DXR-BMD).

Conclusion. Serum MMP-3 and Pyd varied according to disease activity, periarticular osteoporosis measured by DXR, and radiographic outcome, and thus appear to supplement the conventional markers of disease activity for monitoring patients with RA. (J Rheumatol 2004;31:1698–708)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
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MATRIX METALLOPROTEINASES
TYPE I COLLAGEN METABOLISM
TYPE II COLLAGEN METABOLISM

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The destructive potential is a distinctive feature of rheumatoid arthritis (RA), separating it from most other inflammatory joint diseases. The current disease paradigm suggests that persistent synovitis leads to erosive damage indicated by association with markers of disease activity and radiological progression[1-4]. Inflammation measures such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) provide useful information about the general inflammation process, but have proven unspecific and insensitive in monitoring progression in joint destruction and changes in bone mineral density (BMD)[4-7].

Collagen is the most abundant component of the extracellular matrix and it is the structural basis of connective tissues. In the inflammatory rheumatic diseases, the degradation of the collagen fibrils, largely mediated by proteolytic enzymes as metalloproteinases (MMP), is the foundation of irreversible cartilage and bone injuries[8]. The MMP are a family of zinc-dependent endopeptidases that

are capable of degrading all components of the extracellular matrix[8]. In the absence of disease, they take part in normal tissue remodeling, but in pathologic states, they contribute to tissue destruction[8]. MMP-1 (interstitial collagenase) and MMP-3 (stromelysin 1) are thought to be particularly important in arthritis[9-14]. MMP-1 is specific for collagen as substrate. In RA, serum concentrations of MMP-1 have been found, inconsistently, to be elevated in patients with RA and have been associated with disease activity and the development of erosions[10,11]. MMP-3 has a wide variety of connective tissue substrates. Serum and synovial fluid levels of MMP-3 have been correlated with disease activity, ESR, and CRP, but the relationship between MMP-3 levels and radiological joint damage is controversial[12,13]. MMP-13 (collagenase 3), favoring degradation of cartilage collagen type II, has inconsistently been detected in synovial membrane and tenosynovium from patients with RA and has been correlated to disease activity[14-16]. Type I collagen is a major constituent of bone and synovium. The aminoterminal propeptide of type I procollagen (PINP) is a marker of collagen type I formation and has been found to be elevated in patients with RA[17]. In the extracellular matrix, pyridinoline (Pyd) and deoxypyridinoline (DPyd) crosslinks make bridges between adjacent molecules of type I, II, III, V, and XI collagen and are present in bone, synovium, and cartilage in different concentrations[18]. Measurement of urinary levels of Pyd and DPyd has proven useful in the assessment of RA disease activity and changes in BMD[19-22]. In addition to pyridinium crosslinks, crosslinked N-terminal telopeptides and C-terminal telopeptides of type I collagen are excreted.

Recently, a new collagen type I osteoclast-mediated degradation marker, CrossLaps™ (Nordic Bioscience, Copenhagen, Denmark), has been shown to be elevated in patients with active RA and destructive RA[23,24]. However, only scant evidence is available on whether these markers provide information about the metabolism of articular synovium/cartilage in affected joints. For better and earlier evaluation of disease activity and progression as well as treatment effect, biochemical indicators that more specifically reflect the inflammatory synovitis and cartilage and bone injury are required.

In this prospective study, we compared serum concentrations of markers of synovial inflammation, cartilage and bone metabolism in relation to disease activity, BMD of the hand, and radiographic outcome to assess the applicability of the markers of connective tissue metabolism in patients with early RA and unclassified polyarthritis. The biochemical markers of connective tissue metabolism included MMP-1, MMP-3, MMP-13, PINP, Pyd, and CrossLaps™ in serum.

MATERIALS AND METHODS

Seventy-five patients (60 women and 15 men aged 20–82 yrs) with symmetrically swollen and tender second and third metacarpophalangeal (MCP) or proximal interphalangeal (PIP) joints for at least 4 weeks and less than 2 years and who had experienced an effect of nonsteroidal antiinflammatory drug (NSAID) treatment at baseline were studied. All patients were part of an extensive study of consecutive patients with early polyarthritis[25,26]. Patients were followed with monthly intervals up to 2 years. Three patients (one with RA, 2 with unclassified polyarthritis) were excluded from the present study due to other diseases that might influence the measurements (ovarian cancer, hepatitis C, and alcoholic liver disease). At baseline, 44 patients fulfilled the American College Rheumatology (ACR) classification criteria for RA[27], and 7 patients fulfilled the criteria within the first 8 months of the study period. Twenty-one patients had unclassified polyarthritis. There was no significant difference in sex ratio between the 2 groups (Fisher exact test). The F/M ratios were 4:1 for RA patients and 6:1 for polyarthritis patients. Seventeen patients withdrew within the first year and 4 patients within the second year for the following reasons: one RA patient died (pulmonary embolism), 18 patients due to lack of compliance (5 RA and 13 with unclassified polyarthritis), and 2 patients (one RA and one with unclassified polyarthritis) moved to other parts of the country. After one year, 9 patients with unclassified polyarthritis and after 2 years 7 patients were still included in the study. However, 2 withdrawn patients with unclassified polyarthritis returned for the 24-month examination.

Patients with RA were treated according to a strategy aiming at promptly suppressing inflammation with NSAID, disease modifying antirheumatic drugs (DMARD), and glucocorticoids. Ten RA patients were treated with methotrexate (MTX), 39 with sulfazalasine (SSZ), and one with chloroquine. Twenty-seven patients changed DMARD during the study period. Four patients with unclassified polyarthritis were treated with SSZ. Forty-six of 51 RA patients were treated with prednisolone. The cumulated mean dose during the 2-year study period was 1738 ± 357 mg; the mean dose during the first year was 773 ± 145 mg and during the second year 966 ± 218 mg. Ten of the patients with unclassified polyarthritis were treated with glucocorticoids. The cumulated mean dose during the 2-year study period was 351 ± 232 mg; the mean dose during the first year was 172 ± 116 mg and the mean dose during the second year was 179 ± 116 mg.

For the purpose of analysis, patients with RA were divided into 2 groups according to disease activity, based on the measures expressed in time integrated values [area under the curve (AUC); the weighted mean] calculated for each patient during the first 6 months: i.e., active disease defined as ESR > 20 mm/h or CRP > 95 nmol/l and ≥ 1 swollen joint (n = 28); and inactive disease: no swollen joints (n = 22). There was no significant difference in the sex ratio between patients with active and inactive disease. These criteria were chosen because patients with early RA often have only a few swollen joints and normal levels of CRP and ESR.

Irrespective of the classification of the RA patients into groups according to disease activity resulting in different numbers of patients in the groups, all calculations are based on the patients with available radiographs after one year (n = 46) and 2 years (n = 44).

Patients underwent clinical and biochemical investigation every month during the 2-year study period according to the core set of ACR disease activity measures[28,29]. CRP (normal < 95 nmol/l) was determined by nephelometry (Behring-Werke, Germany) and ESR (normal < 20 mm/h) by the Westergren method. Rheumatoid factor (IgM RF) was assessed by nephelometry.

In accord with the Helsinki II Declaration, each patient was informed verbally and in writing about the trial, and all gave their written consent. The study was approved by the local ethical committee.

Biochemical markers of connective tissue metabolism. Blood samples were collected between 8 a.m. and 2 p.m. Serum samples were either analyzed immediately or stored at –80°C until analysis.

MMP-1 in serum was determined by a 2-site sandwich-type ELISA (matrix MMP-1, human Biotrak™ ELISA system, Amersham Pharmacia Biotech). The assay recognizes active MMP-1 and MMP-1/tissue inhibitor of metalloproteinase (TIMP) complexes. According to the manufacturer, the measurement range is 6.25–100 µg/l and the sensitivity 1.7 µg/l. The

median serum MMP-1 in 73 age and sex matched healthy adults (44 women, 29 men, median age 46 yrs, range 19–79) was 6.3 µg/l, and the upper 90th confidence limit was 8.2 µg/l.

MMP-3 in serum was determined by ELISA (matrix MMP-3, human Biotrak™ ELISA system, Amersham Pharmacia Biotech). The assay recognizes pro-MMP-3, active MMP-3, and MMP-3/TIMP complexes. According to the manufacturer, the measurement range is 3.75–120 µg/l and the sensitivity is 2.35 µg/l. The median serum MMP-3 in 73 age and sex matched healthy adults (44 women, 29 men, median age 46 yrs, range 19–79) was 15.8 µg/l and the upper 90th confidence limit was 45.4 µg/l. The serum concentration was approximately 2 times higher in men than in women.

MMP-13 in serum was determined by ELISA (matrix MMP-13, human Biotrak ELISA system, Amersham Pharmacia Biotech) that measures both pro-MMP-13 and active MMP-13. According to the manufacturer, the measurement range is 0.094–3 µg/l and sensitivity is 0.032 µg/l. In 73 age and sex matched controls, MMP-13 could only be detected in 3 specimens; the range was 0.352–0.556 µg/l.

PINP in serum was determined by an ELISA using immunospecifically purified rabbit anti-PINP as the capture antibody and the same antibody conjugated with biotin as an indicator[30]. The detection limit of the PINP ELISA was 62 ng/l. The median serum PINP in 235 healthy adults (127 women, 108 men, median age 48 yrs, range 18–79) was 50 µg/l and the upper 90th confidence limit was 88 µg/l.

Serum Pyd was determined by ELISA (Metra Biosystems, San Diego, CA, USA); the minimum detection limit of the assay is 0.4 nmol/l. According to the manufacturer, the mean level (standard error of the mean, SEM) for healthy men was 1.59 (0.38) nmol/l and for premenopausal women 1.55 (0.26) nmol/l.

The C-telopeptides of type I collagen degradation product were measured as serum CrossLaps™ ELISA (serum CrossLaps™, Nordic Bioscience, Copenhagen, Denmark) based on an isomerized form of a type I collagen-specific sequence (EKAHD-ß-GGR)23. The median serum CrossLaps in 533 healthy adults (419 women, 124 men, median age 55 yrs, range 31–74) was 2.3 µmol/l, and the upper 90th confidence limit was 4.8 µmol/l.

Measurement of BMD by digital x-ray radiogrammetry (DXR). BMD was measured by DXR on standard radiographs of both hands taken every 6 months using the X-posure System™ (Sectra Pronosco A/S, Roskilde, Denmark)[31]. DXR was developed to bridge the gap between radiogrammetry and densitometry[32]. A flatbed scanner was used to record radiographs as digital images (3600 × 3600 dpi, 12 bit resolution). The digitized image is subjected to a number of image-processing algorithms where the 3 regions of interest around the narrowest part of the second, third, and fourth metacarpals are identified automatically[31]. In each region, the cortical thickness and porosity is measured 118 times per cm, symmetrically around the center of the metacarpals. From the cortical thickness, the volume bone per area and a BMD estimate (DXR-BMD) are calculated, together with the average cortical thickness and average metacarpal index (MCI) of the 3 metacarpals. Based on the assumption that the density per volume of bone is constant, the radiogrammetric measurements can be presented as a densitometric estimate (DXR-BMD). For this purpose, the mid-radius region measured by a Hologic QDR-2000 was used as the reference[32]. DXR-BMD is consequently measured in g/cm^2 [31]. The combined cortical thickness of a single metacarpal bone (MCP-CT) was calculated as the sum of the ulnar and radial cortical thicknesses (measured in mm). The metacarpal index of a single joint (MCP-MCI) was calculated as:

(width of bone – combined cortical thickness of bone)/width of bone

The short-term precision, CV%, was previously determined in 40 pre- and postmenopausal women at 0.65%[31].

Radiographs. Radiographs of hands and wrists in posterior-anterior and Nørgaard projection were taken at enrollment and every 6 months during the study period. All available radiographs were read and scored under blind conditions by one experienced radiologist. Each finger and wrist joint was classified as erosive or nonerosive, and each finger and wrist joint was scored according to the Larsen method[33]. Progression was considered to be any magnitude of increase in the Larsen score or the development of bone erosions.

Magnetic resonance imaging (MRI). MRI of the second to fifth MCP joints of the dominant hand was carried out at baseline and at the one-year and 2-year followup visit on a 1.0 T Siemens Magnetom Impact unit (Siemens, Erlangen, Germany) equipped with a receive-only, wraparound surface coil. Continuous axial and coronal T1 weighted spin echo images of the hand (TR/TE/slice thickness 600–700 ms/15 ms/3 mm) were obtained before and after intravenous injection of 0.1 mmol/kg body weight of gadolinium-DTPA (Gd-DTPA, Magnevist, Schering, Berlin, Germany). MR erosions had to be visible on both axial and coronal slices to be diagnosed. The number of MR erosions in each finger joint was counted at baseline and after one and 2 years. Details about the procedure have been reported[25].

Statistical analysis. Analyses were performed using SigmaStat 2.0 (SPSS Inc., Chicago, IL, USA). Because of the skewed distribution, results are expressed as median and range unless otherwise stated. Differences between groups were analyzed by the nonparametric Mann-Whitney rank sum test for unpaired differences. Comparison within groups was by Wilcoxon's test. Correlation analyses were calculated using Spearman's rho test. P values < 0.05 were considered significant. The course of the changes in the variables, e.g., serum CRP, ESR, and swollen joints, were expressed in time-integrated values and the area under the curve (AUC; the weighted mean) was calculated for each patient during the first 6 study months using 7 timepoints (Day 0, 30, 60, 90, 120, 150, 180), during the first 12 months using 13 timepoints (every month), and during the 24 month study period using 25 timepoints (every month)[34]. The course of changes in parameters of connective tissue metabolism (e.g., serum MMP-1, MMP-3, Pyd, PINP, and CrossLaps) was calculated for each patient during the first 6 study months using 4 timepoints (i.e., Day 0, 60, 120, and 180), during the first 12 months using Day 270 and 360, and during the 24 month study using Day 450, 540, 630 and 720, except for serum CrossLaps, measured only at Day 540 and 720 during the last study year.

Serial DXR values for the regions of interest were fitted by linear regression for each subject, and the slope of the regression line (α-coefficient) was used to determine the change of parameters from baseline over 6 months (2 measurements) and over 24 months (5 measurements). The median changes during the first year and total study period (2 years) were calculated for Larsen score (Δ-Larsen score) and DXR-BMD (Δ-DXR-BMD).

RESULTS

Changes in biochemical markers of synovial, cartilage, and bone metabolism according to diagnoses of RA and polyarthritis

Conventional markers of disease activity. Patients' baseline clinical and demographic features according to final diagnoses are presented in Table 1. Patients with RA had significantly higher age and ESR, and more were RF positive than patients with unclassified polyarthritis. Forty-nine percent (25/51) of RA patients and 10% of polyarthritis patients (2/21) had ESR levels > 20 mm/h. No significant difference in CRP level was observed between the 2 groups; however, 41% (21/51) of patients with early RA and 14% (3/21) of patients with unclassified polyarthritis had CRP values > 95 nmol/l. No differences in the number of tender and swollen joints were observed (Table 1).

Patients with early RA had significantly higher average levels of serum CRP and ESR during the first 6 and 12

Table 1. Baseline demographic, clinical, and radiographic characteristics of patients according to the final diagnosis of RA and unclassified polyarthritis. The patients with early RA fulfilled the ACR criteria within one year.

	Polyarthritis, median (range)	RA, median (range)	Difference Between Groups**	Normal Value
No.	21	51		
Sex (F/M)	18/3	41/10	NS	
Age, yrs	39 (27–80)	54 (20–82)	0.004	
Disease duration, mo	3 (1–24)	3 (1–24)	NS	
Swollen joints, n	3 (2–13)	6 (2–18)	NS	
Tender joints, n	16 (2–24)	15 (2–24)	NS	
ESR, mm/h	8 (2–70)	20 (2–105)	0.003	≤ 20
CRP, nmol/l	95 (95–247)	95 (95–1374)	NS	≤ 95
IgM RF, pos/neg	2/19	28/23	0.001	
No. with Larsen > 0	0	13	0.006	
No. with bone erosions	0	10	0.02	
MMP-3, µg/l	11 (4–78)	34* (4–316)	0.01	15.8 (3.8–45.4)
MMP-1, µg/l	12.4* (6.3–16.9)	12.8* (6.3–24.2)	NS	6.3 (6.3–8.2)
PINP, µg/l	61* (17–104)	55 (20–130)	NS	50 (32–88)
Pyd, nmol/l	1.4 (1.0–4.2)	1.8 (0.8–6.1)	NS	1.55† (0.26)
CrossLaps, nmol/l	2.0 (0.6–6.2)	2.6 (0.6–60.0)	NS	2.3 (–4.8)

* Elevated compared to normal values. ** Mann-Whitney rank-sum test for unpaired differences or chi-square test. † mean (SEM).

months of the study period, and also higher ESR levels during the 24 months, than patients with polyarthritis (Table 2). Further, patients with early RA had a significantly higher average number of swollen joints during the study period than patients with unclassified polyarthritis. No difference in the average number of tender joints was observed during the study period (Table 2).

A significant decrease in ESR, but not CRP, at various times during the study period was observed only in early RA patients (Wilcoxon test, data not shown). A significant difference in the percentage change of the acute phase reactants at various times was observed only for ESR (t test). The number of tender and swollen joints in the 2 patient groups decreased significantly during the study period (Wilcoxon test, data not shown), but a significant difference in the percentage change at various times was observed only between the number of swollen joints (t test).

Markers of synovial inflammation and cartilage metabolism. At baseline, patients with RA had significantly higher serum MMP-3 than patients with unclassified polyarthritis and also higher levels compared to age matched controls (Table 1). Thirty-seven percent (19/51) of the early RA patients had elevated serum MMP-3 (i.e., > 45.4 µg/l; 90th percentile of controls), whereas only 5% (1/21) of the patients with unclassified polyarthritis had elevated serum MMP-3 at baseline. No differences in serum MMP-1 were found between patients with early RA and unclassified polyarthritis and controls (Table 1). No significant changes in MMP-3 and MMP-1 were observed during the study period (Wilcoxon test; Figure 1), but the mean level of serum MMP-3 during the first 6 and 12 months was elevated in patients with early RA compared to patients with unclassified polyarthritis (Table 2).

Seven patients (4 with RA) had detectable serum levels

Table 2. Average serum levels of markers of connective tissue metabolism based on the time-integrated values of the first 6, 12, and 24 months for patients with RA and polyarthritis. Values are medians (ranges).

	AUC 6			AUC 12			AUC 24		
	RA	Polyarthritis	p*	RA	Polyarthritis	p*	RA	Polyarthritis	p*
ESR, mm/h	15 (2–94)	7 (4–24)	0.01	14 (2–89)	6 (3–19)	0.02	15 (2–83)	6 (5–13)	0.02
CRP, nmol/l	105 (95–1139)	95 (95–136)	0.02	105 (95–1128)	95 (95–109)	0.03	107 (95–856)	96 (95–102)	NS
Swollen joints, n	3 (0–9)	0 (0–4)	0.007	2 (0–8)	0 (0–2)	0.01	1 (0–7)	0 (0–2)	0.05
Tender joints, n	7 (1–19)	3 (1–15)	NS (0.08)	5 (0–18)	3 (1–13)	NS	3 (0–18)	4 (0–15)	NS
MMP-3, µg/l	44 (4–488)	19 (4–35)	0.04	37 (4–329)	18 (4–33)	NS (0.06)	36 (4–218)	20 (6–31)	NS
MMP-1, µg/l	12.6 (6.3–26.1)	11.7 (6.5–14.2)	NS	12.8 (6.8–27.3)	12.6 (8.6–17.1)	NS	12.3 (7.2–21.8)	11.3 (10.1–15.0)	NS
PINP, µg/l	61 (19–107)	55 (24–113)	NS	65 (19–102)	58 (25–117)	NS	69 (22–123)	48 (32–74)	NS
Pyd, nmol/l	1.6 (1.0–3.9)	1.4 (0.9–2.2)	NS (0.07)	1.6 (1.0–3.5)	1.4 (1.1–2.2)	NS (0.07)	1.6 (1.0–3.6)	1.8 (1.1–1.9)	NS
CrossLaps, nmol/l	3.1 (1.1–26.6)	2.2 (0.5–3.7)	0.03	3.2 (0.9–26.5)	2.6 (0.9–3.8)	NS			

* Mann-Whitney test. NS: nonsignificant.

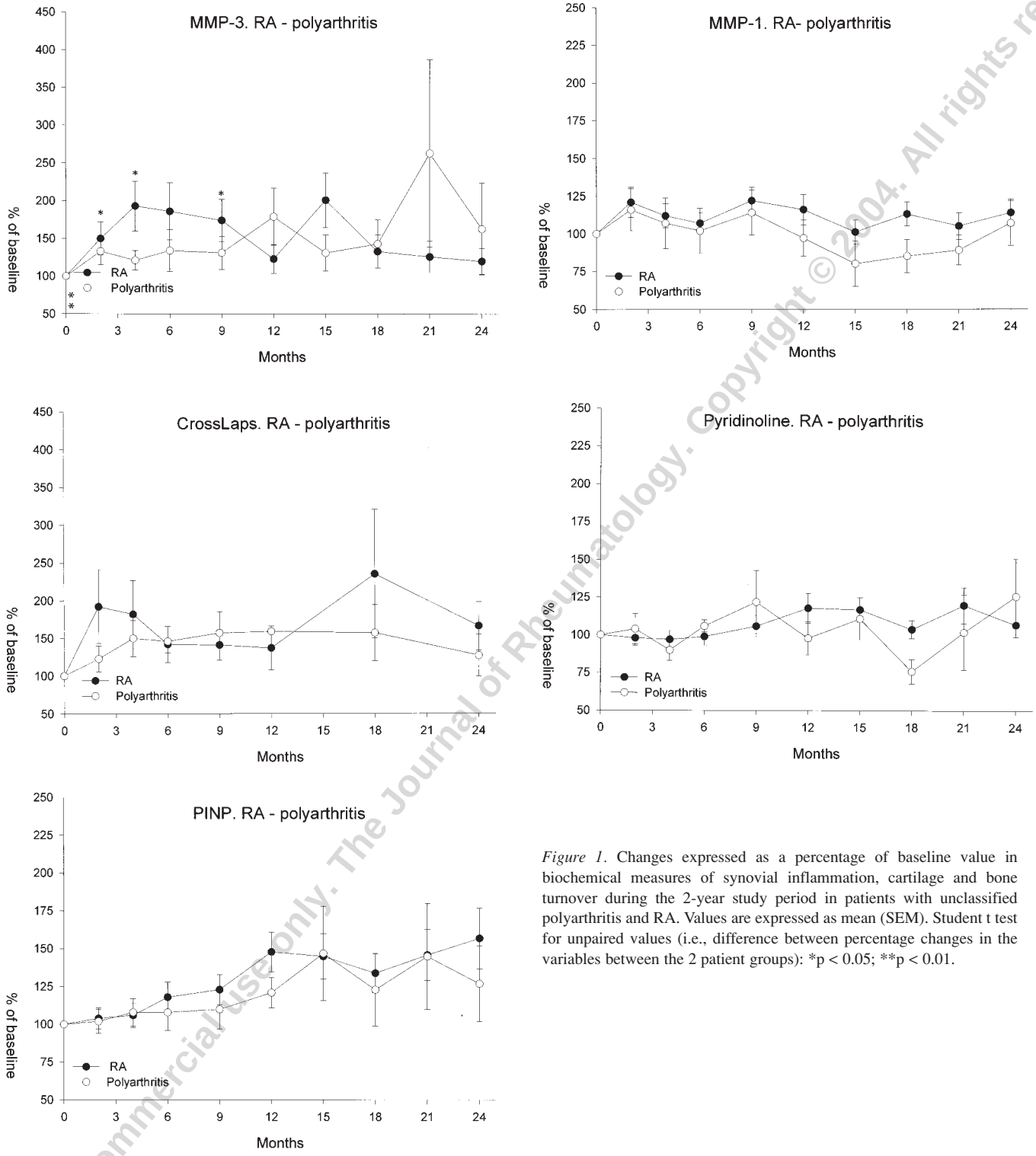

Figure 1. Changes expressed as a percentage of baseline value in biochemical measures of synovial inflammation, cartilage and bone turnover during the 2-year study period in patients with unclassified polyarthritis and RA. Values are expressed as mean (SEM). Student t test for unpaired values (i.e., difference between percentage changes in the variables between the 2 patient groups): *p < 0.05; **p < 0.01.

of MMP-13 (range 0.3–28.2 µg/l). These patients were characterized by more tender (median 20, range 9–24) and swollen joints (median 9, range 2–13) compared to patients with no detectable MMP-13 (medians 14.5, range 2–24, and 4, range 2–18, respectively).

Markers of bone formation and degradation. No differences in serum PINP, Pyd, or CrossLaps levels were found between patients with early RA and unclassified polyarthritis and controls (Table 1). No significant changes in serum PINP, Pyd, or CrossLaps were observed during the

study period (Wilcoxon test; Figure 1), but the mean level of serum Pyd during the first 6 and 12 months tended to be significantly elevated in patients with early RA compared to those with unclassified polyarthritis (Table 2). During the first 6 months, serum CrossLaps was significantly elevated in patients with RA compared to patients with unclassified polyarthritis (Table 2).

Changes in biochemical markers of synovial, cartilage, and bone metabolism in relation to disease activity and mutual relations in patients with RA

Markers of synovial inflammation and cartilage metabolism. No significant changes in serum MMP-3 and MMP-1 levels were observed during the study period in patients with inactive disease at 2 years or in patients who were still active at 2 years (Wilcoxon test, data not shown). Patients with persistently active disease during the first 6 months had significantly higher values of serum MMP-3 than patients with no disease activity, and the differences were also significant when calculated separately for female and male patients with active and inactive RA. No significant differences were observed for serum MMP-1 (Table 3).

Markers of bone formation and degradation. No significant changes in serum PINP, Pyd, or CrossLaps were observed during the study period in patients who were active at 2 years (Wilcoxon test, data not shown). According to disease activity in the first 6 months, patients with active disease had significantly higher levels of serum Pyd than patients with no disease activity, while no differences were detected in serum levels of PINP and CrossLaps (Table 3).

Mutual relations. Mutual correlations based on the time integrated values of 12 and 24 months were observed, but are not shown here, however, as correlation coefficients and p values were almost unchanged (unless otherwise stated) compared to values based on the 6-month AUC (Table 4B).

At baseline, serum MMP-1, MMP-3, and Pyd correlated significantly with swollen joints, and significant mutual correlations between serum MMP-3 and Pyd and CRP and ESR were observed. Serum CrossLaps correlated significantly with ESR (Table 4A).

During the study period, i.e., based on the time-integrated values of 6 months (and 12 and 24 mo), mutual correlations between serum MMP-3 and Pyd and CRP and ESR were noted (Table 4B). Based on 12 and 24-month AUC, serum MMP-3 correlated with swollen joints. Serum MMP-1 and MMP-3 correlated significantly during the first 6 and 12 months (Table 4B). Serum CrossLaps correlated significantly with the disease activity markers ESR (AUC 6, 12, and 24 mo) and CRP (AUC 12 mo, rho = 0.32, $p < 0.05$) and with serum PINP (AUC 6 and 12 mo).

Changes in conventional markers of disease activity and biochemical markers of synovial, cartilage, and bone metabolism in relation to progression in Larsen score, bone erosions, and changes in BMD in patients with RA

At baseline, 13 patients had a Larsen score > 0 (median 10; Table 5) and 10 patients had bone erosions (median 4; Table 5). After 2 years, 22 patients had erosive RA (5 of these patients had erosions detectable only by MRI) and 24 patients had nonerosive RA. No significant difference in the sex ratio was observed between erosive patients and nonerosive patients.

Because analysis of all DXR variables in relation to conventional markers of disease activity and biochemical markers of tissue metabolism gave no further information, only the DXR-BMD results have been described[35].

Conventional markers of disease activity. Neither the baseline values of ESR, CRP, and tender joints (Table 5) nor mean values between the patient groups with erosive disease and nonerosive disease differed during the first 6, 12, and 24 months (Table 6). Patients with erosive disease had significantly higher mean levels of swollen joints during the first 6 and 12 months compared to patients with nonerosive disease (Table 6).

No significant correlations were observed between the conventional markers of disease activity and progression in Δ-Larsen score and Δ-erosions during the first 12 and 24 months (data not shown).

According to changes in BMD, the time-integrated values for ESR (AUC 12) correlated significantly to the α-coefficient (based on 24 mo) of DXR-BMD. Only the time-integrated value of ESR (AUC 12) correlated to the

Table 3. Average serum levels of markers of connective tissue metabolism based on the time-integrated values of the first 6, 12, and 24 months for RA patients with active and inactive disease during the first 6 months of the study period. Values are medians (ranges).

	AUC 6			AUC 12			AUC 24		
	Active Disease	Inactive Disease	p	Active Disease	Inactive Disease	p	Active Disease	Inactive Disease	p
MMP-3, µg/l	72 (3–488)	8 (4–94)	0.001	70 (4–329)	13 (4–188)	0.001	58 (4–188)	18 (3–220)	NS (0.06)
MMP-1, µg/l	11.3 (6.3–26.1)	13.0 (7.2–18.8)	NS	12.2 (6.8–27.3)	13.4 (7.7–15.6)	NS	13.4 (7.2–21.7)	11.3 (7.5–15.5)	NS
PINP, µg/l	61 (32–107)	59 (19–96)	NS	63 (30–102)	65 (19–90)	NS	64 (33–123)	70 (22–95)	NS
Pyd, nmol/l	2.0 (1.0–3.9)	1.5 (1.0–2.3)	0.007	1.8 (1.0–3.5)	1.5 (1.1–2.2)	0.004	1.7 (1.0–3.6)	1.4 (1.2–2.3)	0.008
CrossLaps, nmol/l	3.3 (1.3–13.2)	2.9 (1.1–26.6)	NS	3.5 (1.2–26.5)	2.6 (0.9–4.5)	NS			

* Mann-Whitney test.

Table 4A. Correlation among markers of connective tissue metabolism and other markers of disease activity at study start in RA patients. Values are Spearman rho.

Month 0 Baseline	ESR	Swollen Joints	Tender Joints	MMP-3	MMP-1	Pyd	PINP	CrossLaps
CRP, nmol/l	0.72[c]	0.30[b]	0.15	0.46[c]	–0.15	0.44[c]	0.01	0.14
ESR, mm/h		0.17	0.20	0.45[c]	–0.14	0.41[c]	–0.05	0.28[a]
No. of swollen joints			0.45[c]	0.28[a]	0.33[b]	0.27[a]	–0.04	0.09
No. of tender joints				0.09	0.22	0.06	0.11	0.09
MMP-3, µg/l					–003	0.42[c]	–0.17	0.23
MMP-1, µg/l						0.03	–0.06	0.04
Pyd, nmol/l							0.13	0.07
PINP, µg/l								0.17

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

Table 4B. Correlation among the average levels during the first 6 months (AUC 6 months) of markers of connective tissue metabolism and other markers of disease activity in RA patients. Values are Spearman rho.

AUC 6 Month	ESR	Swollen Joints	Tender Joints	MMP-3	MMP-1	Pyd	PINP	CrossLaps
CRP, nmol/l	0.67[c]	0.23	–0.19	0.50[c]	–0.07	0.43[c]	0.0007	0.25
ESR, mm/h		0.28[a]	0.05	0.42[c]	–0.02	0.43[c]	0.03	0.29[a]
No. of swollen joints			0.54[c]	0.22	0.14	0.05	–0.16	0.15
No. of tender joints				0.02	0.05	–0.134	–0.27[a]	–0.16
MMP-3, µg/l					0.27[a]	0.42[c]	–0.23	0.15
MMP-1, µg/l						0.11	–0.18	0.15
Pyd, nmol/l							0.11	0.12
PINP, µg/l								0.40[b]

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

Table 5. Demographic, clinical, and radiographic characteristics of RA patients according to erosive and nonerosive RA status at start of study. The patients with early RA fulfilled the ACR criteria within one year.

	Erosive RA, median (range)	Nonerosive RA, median (range)	Difference Between Groups**
No.	22	24	
Age, yrs	57 (29–82)	50 (20–82)	0.04
Disease duration, mo	3 (1–22)	4 (1–24)	NS
Swollen joints, n	7 (2–15)	5 (2–18)	NS
Tender joints, n	17 (2–24)	15 (2–22)	NS
ESR, mm/h	20 (3–50)	16 (2–96)	NS
CRP, nmol/l	95 (95–1365)	95 (95–1374)	NS
IgM RF, pos/neg	11/11	12/12	NS
Larsen score	10 (0–30)	0 (0–2)	
MMP-3, µg/l	42* (4–304)	21 (4–227)	NS (0.06)
MMP-1, µg/l	14.2* (6.3–24.2)	12.8 (6.3–23.3)	NS
PINP, µg/l	68* (25–130)	48 (20–70)	< 0.001
Pyd, nmol/l	1.9 (1.3–3.3)	1.7 (0.8–4.0)	NS
CrossLaps, nmol/l	2.8 (0.8–60.0)	2.5 (0.6–9.5)	NS

* Elevated compared to normal levels. ** Mann-Whitney rank-sum test for unpaired differences or chi-square test.

Δ-DXR-BMD change during the 2-year study period (Table 8).

Markers of synovial inflammation and cartilage metabolism. Baseline MMP-3 and mean levels of MMP-3 during the first 6, 12, and 24 months were significantly higher in patients who developed erosive RA compared to patients who remained nonerosive during the study period (Table 6). A significant difference was still observed when calculated

Table 6. Average serum levels of markers of connective tissue metabolism based on the time-integrated values of the first 6, 12, and 24 months for RA patients with erosive and nonerosive RA. Values are medians (ranges).

	AUC 6			AUC 12			AUC 24		
	Erosive RA	Nonerosive RA	p*	Erosive RA	Nonerosive RA	p*	Erosive RA	Nonerosive RA	p*
ESR, mm/h	20 (4–82)	13 (2–94)	NS	16 (3–84)	10 (2–89)	NS	15 (3–75)	15 (2–83)	NS
CRP, nmol/l	116 (95–938)	99 (95–1139)	NS	121 (95–1067)	105 (95–1128)	NS	116 (95–708)	104 (95–856)	NS
Swollen joints, n	3 (0–8)	2 (0–8)	0.05	2 (0–5)	1 (0–8)	0.01	2 (0–7)	1 (0–4)	NS
Tender joints, n	6 (1–16)	6 (1–19)	NS	5 (0–16)	5 (0–18)	NS	3 (1–18)	3 (0–11)	NS
MMP-3, µg/l	68 (5–488)	15 (4–161)	0.02	55 (5–329)	21 (4–190)	0.006	49 (5–220)	16 (4–188)	0.02
MMP-1, µg/l	13.5 (9.0–21.5)	12.5 (6.3–26.1)	NS	12.7 (8.8–23.9)	12.9 (6.8–27.3)	NS	12.4 (7.6–21.7)	11.6 (7.2–21.8)	NS
PINP, µg/l	61 (32–107)	59 (19–96)	NS	67 (30–102)	61 (19–97)	NS	71 (33–101)	68 (22–123)	NS
Pyd, nmol/l	1.7 (1.2–3.5)	1.5 (1.0–3.9)	NS (0.07)	1.7 (1.3–3.1)	1.5 (1.0–3.5)	NS (0.09)	1.7 (1.2–3.6)	1.5 (1.0–3.4)	NS
CrossLaps, nmol/l	3.6 (1.1–13.2)	3.1 (1.2–26.6)	NS	3.5 (1.2–8.7)	3.2 (0.9–26.6)	NS			

* Mann-Whitney test.

Table 7. Correlation coefficients among markers of connective tissue metabolism, other markers of disease activity, and α-coefficient DXR-BMD of the left hand during the 2-year study period. RA patients only. Values are Spearman rho.

	Month 0	AUC 6 Months	AUC 12 Months	AUC 24 Months
CRP, nmol/l	–0.10	–0.21	–0.21	–0.12
ESR, mm/h	–0.16	0.09	–0.29[a]	–0.24
No. of swollen joints	–0.03	–0.16	–0.11	–0.22
No. of tender joints	–0.05	0.04	–0.04	–0.16
MMP-3, µg/l	–0.35[a]	–0.36[a]	–0.42[b]	–0.37[a]
MMP-1, µg/l	–0.16	–0.13	–0.18	–0.18
Pyd, nmol/l	–0.35[a]	–0.43[b]	–0.49[c]	–0.47[b]
PINP, µg/l	–0.14	–0.23	–0.27	–0.34[a]
CrossLaps, nmol/l	–0.40[b]	–0.08	–0.09	—

[a:] $p < 0.05$; [b:] $p < 0.01$; [c:] $p < 0.001$.

Table 8. Correlation coefficients among markers of connective tissue metabolism, other markers of disease activity, and the changes in Δ-DXR-BMD of the left hand during the 2 year study period. RA patients only. Values are Spearman rho.

	Month 0	AUC 6 Months	AUC 12 Months	AUC 24 Months
CRP, nmol/l	–0.11	–0.23	–0.27	–0.19
ESR, mm/h	–0.21	0.12	–0.34[a]	–0.28
No. of swollen joints	–0.06	–0.28	–0.23	–0.29
No. of tender joints	–0.05	–0.02	–0.04	–0.12
MMP-3, µg/l	–0.40[b]	–0.41[b]	–0.49[c]	–0.41[b]
MMP-1, µg/l	–0.07	–0.13	–0.17	–0.20
Pyd, nmol/l	–0.37[a]	–0.45[b]	–0.56[c]	–0.56[c]
PINP, µg/l	–0.07	–0.14	–0.23	–0.27
CrossLaps, nmol/l	–0.37[b]	–0.15	–0.16	—

[a:] $p < 0.05$; [b:] $p < 0.01$; [c:] $p < 0.001$.

for men only, whereas no significant difference was found between erosive and nonerosive female patients, although a tendency to elevated serum MMP-3 levels in female patients with erosive disease was observed (data not shown). MMP-1 did not differ significantly between the groups. MMP-3 and MMP-1 did not change significantly during the study period in these 2 patient groups (Wilcoxon test, data not shown).

No differences in the progression in Δ-Larsen score or Δ-erosions appeared among patients with elevated baseline levels or persistently elevated levels of MMP-3 and MMP-1 (i.e., greater than median level: MMP-3 > 37 µg/l and MMP-1 > 12.8 µg/l based on AUC during the first 12 mo) during the first 6, 12, and 24 months of the study period (data not shown).

No correlations with the progression in Larsen score

were observed for serum MMP-1 and MMP-3, whereas a significant correlation was found between the time-integrated values of serum MMP-3 (AUC 12 and 24 mo) and the progression in erosions during the 2-year study period (rho = 0.31 and rho = 0.30, $p < 0.05$).

The baseline values and the time-integrated values of serum MMP-3 (AUC 6, 12, and 24 mo) correlated significantly to the α-coefficient of the DXR-BMD of the left hand (Table 8). According to the annual changes in Δ-BMD, the baseline values and the AUC 6, 12, and 24 month values of serum MMP-3 correlated to the change in Δ-DXR-BMD during the 2-year study period (Table 7).

Markers of bone formation and degradation. Patients with erosive disease had significantly higher levels of serum PINP at study start than patients without erosions (Table 5). Tendencies were observed toward higher serum Pyd at baseline (Table 5) and during the first 6 and 12 months (Table 6) in patients who developed erosive disease. No differences in the progression of Δ-Larsen score or Δ-erosions were observed among patients with persistently elevated levels of serum PINP, Pyd, and CrossLaps during the first 6, 12, and 24 months (median values of markers based on calculation of AUC during the first 12 mo; data not shown).

Only the baseline value of serum PINP showed a significant relationship to progression in Δ-Larsen score during the first 12 and 24 months (rho = 0.30 and rho = 0.30, $p < 0.05$).

The baseline value of CrossLaps and the time-integrated values of Pyd (AUC 6, 12, and 24 mo) and PINP (AUC 24 mo) correlated significantly to the a-coefficient of the DXR-BMD of the left hand (Table 8). According to the annual changes in Δ-DXR-BMD, the baseline values of serum Pyd and CrossLaps and the AUC 6, 12, and 24 month values of serum Pyd correlated with the change in Δ-DXR-BMD during the 2-year study period (Table 7).

DISCUSSION

RA is a complex and heterogeneous disease affecting the synovial membrane, cartilage, and periarticular bone. The markers of disease activity currently used in RA, clinical assessment, the acute phase reactants ESR and CRP, and radiology, reveal the pathological processes in the inflamed joints only indirectly, and may be negative at disease onset[36-38]. The development of joint erosions is unpredictable and may continue despite effective suppression of inflammation[3,39]. Consequently, new markers reflecting the metabolic and pathologic processes in the joints may provide more specific information on disease activity and radiological progression.

Unclassified polyarthritis and early RA. No differences in the conventional biochemical markers of disease activity, except for ESR, were observed at study start among our patients with early RA and unclassified polyarthritis. However, during the first study year, the average levels of the conventional measures of disease activity were higher in RA patients, concordant with persistent disease activity, in contrast to unclassified polyarthritis. Although a tendency to elevated serum Pyd was also observed, only the initial and average serum MMP-3 was higher during the first study year in the RA group. MMP-13 was only detectable in a few patients. Recently, it has been reported that initial levels of serum MMP-1 and MMP-3 were elevated in RA patients compared to patients with unclassified polyarthritis, but in contrast to our observations, the time integrated levels of serum MMP-1, but not MMP-3, were elevated[10]. However, this difference may be a consequence of the different assay systems. The majority of MMP in sera are present as their precursor forms, and the applied assay detects active MMP-1 and MMP-1/TIMP complexes. The (often subclinically sustained) inflammation in RA may be reflected in the elevated serum levels of MMP-3 and Pyd. Measurement of serum MMP-3 may supplement the conventional markers of disease activity in differentiating between unclassified arthritis and early RA, but it has no predictive value in itself.

RA patients. Markers of synovial, cartilage, and bone metabolism. The central observations in this study involved the relationships between serum levels of MMP-3 and disease activity and erosive disease. The positive relationship between serum levels of MMP-3 and markers of joint inflammation (ESR, CRP) supports data from previous studies[10,40]. Increased MMP-3 levels in synovial fluid are found in inflammatory arthropathies and so are not specific to erosive joint diseases[10,12]. The relationship between MMP-3 levels and the development of joint destruction is controversial[10,14,41,42]. However, baseline levels of MMP-3 have been associated with progression in Larsen score in patients with early RA, as levels of MMP-3 have been correlated with joint space narrowing, in accord with the fact that the main targets of MMP-3 are localized in the cartilage matrix. Further, the association of MMP-3 with changes in DXR-BMD indicates a link between synovial inflammation and degradation of cartilage and subchondral bone and periarticular osteoporosis. Since the destructive potential of MMP-3 is dependent on its activation, the nonstandardized assays measuring different aspects of MMP-3 (pro-MMP-3, complexed, active MMP-3) contribute to the contradictory results for the role of MMP-3 in joint destruction. Further, the differences may be explained by the sex-dependent levels of serum MMP-3, which often have not been taken into account[43].

Although MMP-1 was elevated in patients with RA, no differences were observed between patient groups with active and inactive disease or with and without erosions. Inconsistently, the serum levels of MMP-1 have been found to be elevated in RA patients, associated with disease activity and erosive disease[10,11,14]. Serum MMP-1 appeared, in this study, to be less sensitive as a marker of disease activity and erosive disease.

In this study Pyd was associated with disease activity, erosive disease, and changes in hand BMD. The relationship to disease activity is consistent with previous studies of urinary Pyd and serum Pyd[21,22,44]. Previous studies of RA and Pyd focused on Pyd in relation to BMD. A significant association of axial BMD and serum Pyd has been described, in accord with the hypothesis that bone resorption is the principal mechanism of secondary osteoporosis in RA[21,44]. Pyd occurs in cartilage as well, in contrast to the almost exclusive distribution of DPyd in bone[17]. Urinary Pyd and serum Pyd correlated significantly with synovial fluid Pyd in patients with RA[20,22]. In this study, decrease in appendicular BMD, i.e., in hand DXR-BMD, correlated negatively to Pyd. However, no significant associations to markers of bone formation and degradation were observed. These observations suggest that a considerable amount of serum Pyd is derived from the articular compartments, including synovium, in addition to systemic bone. Further, the positive correlations of Pyd to indices of disease activity, MMP-3, and periarticular osteoporosis underscore the hypothesis that serum Pyd predominantly reflects the degradation of cartilage and subchondral bone in patients with RA.

Type I collagen is a major constituent of bone and synovium, but is also present in tendons and ligaments. Thus a contribution from other tissues than bone to the circulating levels of type I collagen markers is possible. The baseline value of PINP, but not the mean level of PINP during the study period, was elevated in patients with RA and erosive disease compared to normal values. Elevated serum levels of PINP have been found in patients with RA[17], and the abundance of PINP in synovial fluid observed in patients with RA probably reflects the increased formation in parallel to increased degradation of type I collagen in the inflamed synovium and subchondral bone[17]. These observations are in accord with the relationship of elevated PINP and progression in radiological score, but not to changes in axial BMD[45]. However, 46 of 51 patients were treated with glucocorticoid and the glucocorticoid-induced suppression of inflammation, cell proliferation, and synthesis of proteins, including type I collagen, result in decreased levels of PINP. This may explain our observation of only initial but not persistently elevated serum PINP. The average serum levels of CrossLaps were elevated in patients with RA compared to patients with undifferentiated polyarthritis. A relationship to measures of disease activity, but not to bone changes, was observed. The latter contrasts with the few earlier observations on serum CrossLaps that have shown a relationship to disease activity and erosive RA[24]. The markers of type I collagen turnover in serum may reflect joint turnover as well as general bone turnover, thus blurring the focus.

Serum MMP-3 and Pyd varied according to disease activity, periarticular osteoporosis measured by DXR, and progression in Larsen score. These biochemical markers, Pyd and especially MMP-3, may be more specific, local, and sensitive markers of ongoing inflammation than ESR and CRP; and they seem to be valuable tools in identifying patients with RA and monitoring disease activity and treatment efficacy. However, studies of large populations characterized by homogenous disease activity and treatment are needed to test these hypotheses.

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